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(12) **United States Plant Patent**
Naganuma et al.

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(54) **MUSHROOM NAMED 'HOKUTO NT-100'**

(50) Latin Name: ***Grifola frondosa***
Varietal Denomination: **Hokuto NT-100**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 69 days.

(21) Appl. No.: **11/004,584**

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(65) **Prior Publication Data**

US 2006/0162028 P1 Jul. 20, 2006

(51) **Int. Cl.**
A01H 15/00 (2006.01)

(52) **U.S. Cl.** **Plt./394**

(58) **Field of Classification Search** Plt./394
See application file for complete search history.

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(57) **ABSTRACT**

A new and distinct variety of mushroom, named Hokuto NT-100, is described, which mushroom has the following characteristics: large cap, thick meat, nice consistency, nice taste, uniform growth when cultivated in a facility, and suitable for bottle cultivation. The variety of mushroom named Hokuto NT-100 was developed from mushrooms related to Hokuto MY-95 and Hokuto MY-75, which are registered varieties of a Japanese corporation and a wild variety found in Aomori Prefecture of Japan.

13 Drawing Sheets

BRIEF SUMMARY OF THE INVENTION

The present invention relates to a new and distinct variety of mushroom having the following characteristics: large cap, thick meat, nice consistency, nice taste, uniform growth when cultivated in a facility, and suitable for bottle cultivation. The new variety of mushroom named Hokuto NT-100 was developed from mushrooms related to Hokuto MY-95 and Hokuto MY-75, which are registered varieties of a Japanese corporation, and a wild variety found in Aomori Prefecture of Japan.

Latin name of genus and species and variety denomination of the claimed plant: The Latin name of the genus and species of 'Hokuto NT-100 mushroom' is *Grifola frondosa* (Dicks, ex Fr.) S. F. Gray, this mushroom is generally called "Maitake" in Japan and seems to be called "hen of the woods" in English.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a top view of the Hokuto NT-100 mushroom;

FIG. **2** is an oblique view of the Hokuto NT-100 mushroom;

FIG. **3** is a partial side view of the Hokuto NT-100 mushroom;

FIG. **4** is an oblique view of the Hokuto NT-100 mushroom in bottle cultivation;

FIG. **5** is a side view of the Hokuto NT-100 mushroom in bottle cultivation;

FIG. **6** is a top view of the Hokuto NT-100 mushroom in outdoor cultivation;

FIG. **7** is a top view of the Hokuto MY-95 mushroom for the purposes of comparison;

FIG. **8** is a side view of the Hokuto MY-95 mushroom;

FIG. **9** is a top view of the Hokuto MY-95 mushroom in outdoor cultivation;

FIG. **10** is a top view of the MH18211 mushroom for the purposes of comparison;

FIG. **11** is a side view of the MH18211 mushroom;

FIG. **12** is a top of the MH18211 mushroom in outdoor cultivation, and

FIG. **13** is a chart showing the system which resulted in the Hokuto NT-100 mushroom.

DETAILED DESCRIPTION OF THE INVENTION

The history of the Hokuto NT-100 mushroom in terms of improvement period and the like are set forth in the following chronological list of each stage of variety improvement:

January 1993: By crossing MH18206 amd MH18215, the following three fugal strains were obtained: Hokuto MY-75, Hokuto MY-95 and GD99.

February 1995: Hyphae were obtained from Hokuto MY-75 and GD99.

March 1995: By crossing the hyphae of Hokuto MY-75 and GD99, MH182114 was obtained.

May 1996: From Hokuto MY-95, hyphae were obtained.

June 2000: From MH18284, hyphae were obtained.

August 2000: By crossing the hyphae of MH18284 and MY-95, MH182113 was obtained.

November 2000: From MH182114, hyphae were obtained.

July 2001: From MH182113, hyphae were obtained.

January 2002: By crossing the hyphae of MH182113 and MH182114, a superior variety MH182115 was obtained.

March 2002 to March 2003: Tests were repeated to identify the characteristics of MH182115, and during this time, it was found that bottle cultivation was favorable, and stability, uniformity and differentiability were confirmed. In November 2002, MH182115 was named Hokuto NT-100 and variety improvement was completed. More tests were conducted after that, and characteristics tests

were completed in March 2003. The claimed cultivar is stable and reproduces true to type in successive generations of asexual reproduction.

The system which resulted in the Hokuto NT-100 mushroom is shown in the flow chart of FIG. **13**.

The Hokuto NT-100 mushroom has the following characteristics: large cap, thick meat, nice consistency, nice taste, uniform growth when cultivated in a facility, and suitable for bottle cultivation.

The differences between the new variety and its parents are as follows:

The parent varieties are MH182113 and MH182114. Both the parent varieties; and the instant new variety of this application can be defined being different genetically because they show a positive reaction in a dual culture. The new variety is different from MH182113 in cultural characteristics in that the new variety can be yielded numerously and has a different cap color. The new variety differs from MH182114 in yield, cap color and number of cilia on the cap. The differences therebetween are summarized in the following table.

TABLE 1

Morphological Differences	Instant New Variety	MH182113	MH182114
Yield (grams)	547.1	350.0	787.8
Cilia on the cap	Small	Small	Dark gray-brown Large

When Hokuto NT-100 was cultured with commercially available varieties, inhibition zones were seen. In addition, the optimal temperature for hyphal growth was approximately 24 degree C.

The morphological characteristics of Hokuto NT-100 are as follows: at the time of harvest, the cap is fan-shaped or circular; the margin is rounded off; hair is minimal; pore shape is elliptical (Type 1); and pore cross section is uneven. When naturally cultivated, the mode of development is medium and development occurs in September. Experimental data have also shown that the length of time from inoculation to development is 63 days; the optimal development temperatures is 18–20 degree C.; the optimal growth temperature is 17–18 degree C.; and yield is 547.2 g.

With regard to other characteristics, the vitamin D_2 content of Hokuto NT-100 was high at 20.6 mg/100 g (international units: 824 IU) according to experimental data.

Some of these characteristics tests for Hokuto NT-100 as compared to similar mushroom varieties Hokuto MY-95 and MH18211 (Mori Sangyo #51) are summarized in the following table:

TABLE 2

	Characteristic code of filed variety				
Characteristics	01	02	03	04	05
Genetic					
Dual culture					
Dislike-touch reaction					
Physiology					
Mycelial growth					
Effect of temperature					
The optimum (° C.)	22	23	24	25	26
Growth rate					
10° C.					
15° C.					
20° C.					
25° C.					
30° C.					
35° C.					
Mycelial color	white				
shape of the surface of the colony	smooth	tough			
Shape of colony edge	regular				
colony thickness			thin		medium
Density of hyphae			sparse		medium
Cultivation characteristics					
Capsule formation			little		medium
Capsule coloration	Absent				
Length of time until fruit body fromation at optimal temperature	<30	31~40	41~50	51~60	61~0
Development period (natural cultivation)					
Optimal development temperature (C.)	<15	15~20	20~29	>25	
Optimal fruit body growth temperature (C.)	<18	18~21	>21		
Other					
Budding period (natural cultivation)			Early		inter-mediate
Yield (g/bottle)					
Morphology					
Cap					
Size			small		medium
Thickness			Thin		medium
Notch	Absent				
Cross sectional shape	1	2	3		
Face color	W	G-W	D-G	B	D-B
Annulus	1	2	3	4	
Hair			fair		medium
Tube					
Shape of tube face	1	2	3	4	
Unevenness of cross sectional the tip of tube	Absent				
Tube position	1	2	3		
Stipe shape	1	2	3		

	Characteristic code of filed variety				Note
Characteristics	06	07	08	09	(Data)
Genetic					
Dual culture					
Dislike-touch reaction				+	–
Physiology					
Mycelial growth					
Effect of temperature					
The optimum (° C.)	27	28	29	30	24° C.
Growth rate					
10° C.					0.8
15° C.					1.7
20° C.					2.4
25° C.					2.6
30° C.					1.0
35° C.					0.1
Mycelial color				other	white
shape of the surface of the colony				other	smooth
Shape of colony edge				regular	irregular
colony thickness		thick			thick
Density of hyphae		dense			sparse
Cultivation characteristics					

TABLE 2-continued

Capsule formation	much		medium
Capsule coloration		present	absent
Length of time until fruit body fromation at optimal temperature	>70		63 days
Development period (natural cultivation)			6 month
Optimal development temperature (C.)			18~20° C.
Optimal fruit body growth temperature (C.)			17~18° C.
Other			
Budding period (natural cultivation)	Late		intermediate
Yield (g/bottle)			547.2 g
Morphology			
Cap			
Size	large		56.4 mm
Thickness	Thick		2.6 mm
Notch		present	Absent
Cross sectional shape			type 2
Face color		other	dark Brown
Annulus		other	type 3
Hair	many		few
Tube			
Shape of tube face		other	type 1
Unevenness of cross sectional the tip of tube		present	present
Tube position		other	type 3
Stipe shape		other	type 2

Characteristics	Code of the similar variety: Hokuto MY-95	HM18211
Genetic		
Dual culture		
Dislike-touch reaction	09	09
Physiology		
Mycelial growth		
Effect of temperature		
The optimum (° C.)	04	05
Growth rate		
10° C.	0.9	0.8
15° C.	1.4	1.8
20° C.	1.6	2.5
25° C.	2.0	2.9
30° C.	1.2	2.7
35° C.	0.1	0.2
Mycelial color	01	01
shape of the surface of the colony	01	01
Shape of colony edge	01	09
colony thickness	05	03
Density of hyphae	03	03
Cultivation characteristics		
Capsule formation	05	05
Capsule coloration	01	09
Length of time until fruit body fromation at optimal temperature	05	06
Development period (natural cultivation)	6 month	7 month
Optimal development temperature (C.)	02	02
Optimal fruit body growth temperature (C.)	01	01
Other		
Buding period (natural cultivation)	05	05
Yield (g/bottle)	575.9 g	338.9 g

TABLE 2-continued

Morphology		
Cap		
Size	07	07
Thickness	05	05
Notch	01	09
Cross sectional shape	02	02
Face color	Grayish brown	Grayish yellow
Annulus	03	02
Hair	03	03
Tube		
Shape of tube face	02	01
Unevenness of cross sectional the tip of tube	09	09
Tube position	03	03
Stipe shape	02	01

The numbers used to describe the characteristics of the cross-sectional shape, annulus, shape of tube face and the others of the instant new variety are indicative of the morphological types prescribed in the Examination Standard stipulated officially in Japan. Distinguishable characteristics of Hokuto NT-100 are set forth in the following:

When cultured with Hokuto MY-95, an inhibition zone is seen. Hokuto NT-100 is different from Hokuto MY-95 in the following characteristics: optimal growth temperature is 24 and 26 degree C., respectively; meat thickness is thick and regular, respectively; yield is 547.2 and 575.9 g, respectively; cap surface color is dark red and grayish brown, respectively; and pore surface shape is type 1 and 2 prescribed in the Examination Standard officially stipulated in Japan, respectively. When cultured with MH18211, an inhibition zone is seen. Hokuto NT-100 is different from MH18211 in the following characteristics: optimal growth temperature is 24 and 28 degree C., respectively; margin shape is irregular and regular, respectively; meat thickness is thick and regular, respectively; bottom capsule is present and absent, respectively; length of time from inoculation to development is 63 and 73 days, respectively; length of time for natural development is 6 and 7 months, respectively; yield is 547.2 and 338.9 g, respectively; cap gill is present and absent, respectively; cap surface is color dark red and grayish yellow, respectively; annellation type is 3 and 2, respectively; and cross section shape of stipe is type 2 and 1, respectively.

The above comparative test results were based, at least in part, on the following tests where the cultivation conditions under which the above-mentioned characteristic tests were conducted were as follows:

a. Cultivation site: Nagano, Japan.

b. Cultivation period: March 2002 to March 2003.

c. Cultivation methods (location, facility, medium, cultivation mode, scale, etc.). Mushrooms listed in the test results were cultivated using conventional methods in a culture room with 300 containers and a growth room with 300 containers. In the characteristics tests, cultivation was performed using ten bags per block and the cultivation experiment was repeated twice.

The following tests were conducted:

(*1*) *Dual culture.*—Conducted according to the examination standards for mushrooms where characteristics were observed on Difco PDA medium. The strains used were: MH18211 Commercial strain (Mori Sangyo #51). MH182113 mating strain. MH182114 mating strain. MH18284 Wild type. Hokuto MY-75. Hokuto MY-95. MH18212 Com-

mercial strain (Fujimoto sangyou M10). MH18222 Commercial strain (Tochiokimaitake). MH148261 Commercial strain (Tokatiana). With regard to the dislike-touch reaction, the results were as follows where + is present and − is absent.

TABLE 3

	Hokuto NT-100
Hokuto NT-100	−
MH18211	+
MH182113	+
MH182114	+
MH18284	+
Hokuto MY-75	+
Hokuto MY-95	+
MH18212	+
MH18222	+
MH148261	+

(2) *Mycelial growth.*—The growth of Hokuto NT-100 was compared with Hokuto MY-95 and MH18211.

TABLE 4

temperature (° C.)	10	15	20	25	30	35
Hokuto NT-100 (mm/day)	0.8	1.7	2.4	2.6	1.0	0.1
Hokuto MY-95 (mm-day)	0.9	1.4	1.6	2.0	1.2	0.1
MH18211 (min/day)	0.8	1.8	2.5	2.9	2.7	0.2

TABLE 5

temperature (° C.)	20	22	24	26	28	30	optimum temp.
Hokuto NT-100 (mm/day)	2.3	2.5	2.6	2.5	2.1	1.3	24
Hokuto MY-95 (mm-day)	1.6	1.8	2.1	2.2	2.0	1.9	26
MH18211 (mm/day)	2.4	2.8	3.0	3.2	3.3	3.2	28

(3) *Characteristics data.*—Cultivation data for Hokuto NT-100 was compared with Hokuto MY-95 and MH18211.

TABLE 6

Characteristics	Hokuto NT-100	Hokuto MV-95
Capsule formation	Medium	Medium
Capsule coloration	Absent	Absent
Length of time until fruit body formation at optimal temperature	60-63 days	60-63 days
Optimal development temperature	18-21 C.	18-21 C.
Optimal fruit body growth temperature	17-18 C.	17-18 C.
Development period (natural cultivation)	September	September
Yield	574.2 ± 2.6 g	575.9 ± 53.8 g
Cap size	56.4 ± 16.5 mm	43.6 ± 15.8 mm
Cap thickness	2.6 ± 0.4 mm	2.6 ± 0.5 mm
Cap surface color	Dark yellowish red (1910)	Grayish brown (1919)

Characteristics	MH18211
Capsule formation	Medium
Capsule coloration	Present
Length of time until fruit body formation at optimal temperature	71-73 days
Optimal development temperarure	18-20 C.
Optimal fruit body growth temperature	17-18 C.
Development period (natural cultivation)	September
Yield	338.9 ± 44.7 g
Cap size	45.1 ± 10.9 mm
Cap thickness	2.6 ± 0.5 mm
Cap surface color	Grayish yellow (2212)

(4) *Temperature data.*—Data for the optimum development temperature of Hokuto NT-100 was determined using conventional cultivation methods where fungal development was monitored at different temperatures, the results being as follows:

TABLE 7

Primordium formation at each development temperature

Development temperature (C.)	Hokuto NT-100
<15	Primordium formation occurred 61 days after inoculation
16-20	Primordium formation occurred 56 days after inoculation
21-25	Low pigmentation on the culture medium surface
>25	No budding

As shown in Table 7, while primordium formation occurred at temperatures below 20° C., when considering cultivation style and productivity, earlier primordium formation is desirable. Therefore temperatures between 16 and 20° C. were suitable, and, in this temperature range, development was most favorable at 18–20° C.

(4) Vitamin D2 Content.

The vitamin D2 content in the fruit body was measured with HPLC and the results were as follows:

TABLE 8

	Hokuto NT-100	Hokuto MY-95	HM18211
The vitamine D2(μg/100 g)	20.6	15.6	7.1
The vitamine D2(IU/100 g)	824.0	624.0	280.0

With regard to asexual reproduction of the subject mushroom:

(1) The fungal strains in question are preserved at 5° C. on agar slant culture (agar medium) and continuously cultivated once a year.

(2) The medium is a potato dextrose agar culture (PDA) made of 200 grams of potato, 20 grams of glucose, and 15 grams of agar so as to have a pH value of 5.5 in 1 liter of distilled water.

(3) The cultivation of strains is carried out by picking out mycelial fragment from the fungal strains preserved in (1) mentioned above and put on the medium of (2). Then, the strains are cultivated at about 25° C. for 14 days. After bacterial threads of the strains preserved, the strains are displanted to an incubation medium.

In a case of preserving the strains once again, the strains thus cultivated may be restored to the process (1) above.

The characteristic colors of parts of the instant new variety are recited herein based on Japan Color Standard for Horticultural Plant (JHS Color Chart).

It is claimed:

1. A new and distinct variety of mushroom, substantially as described and illustrated herein.

* * * * *

FIG. 1

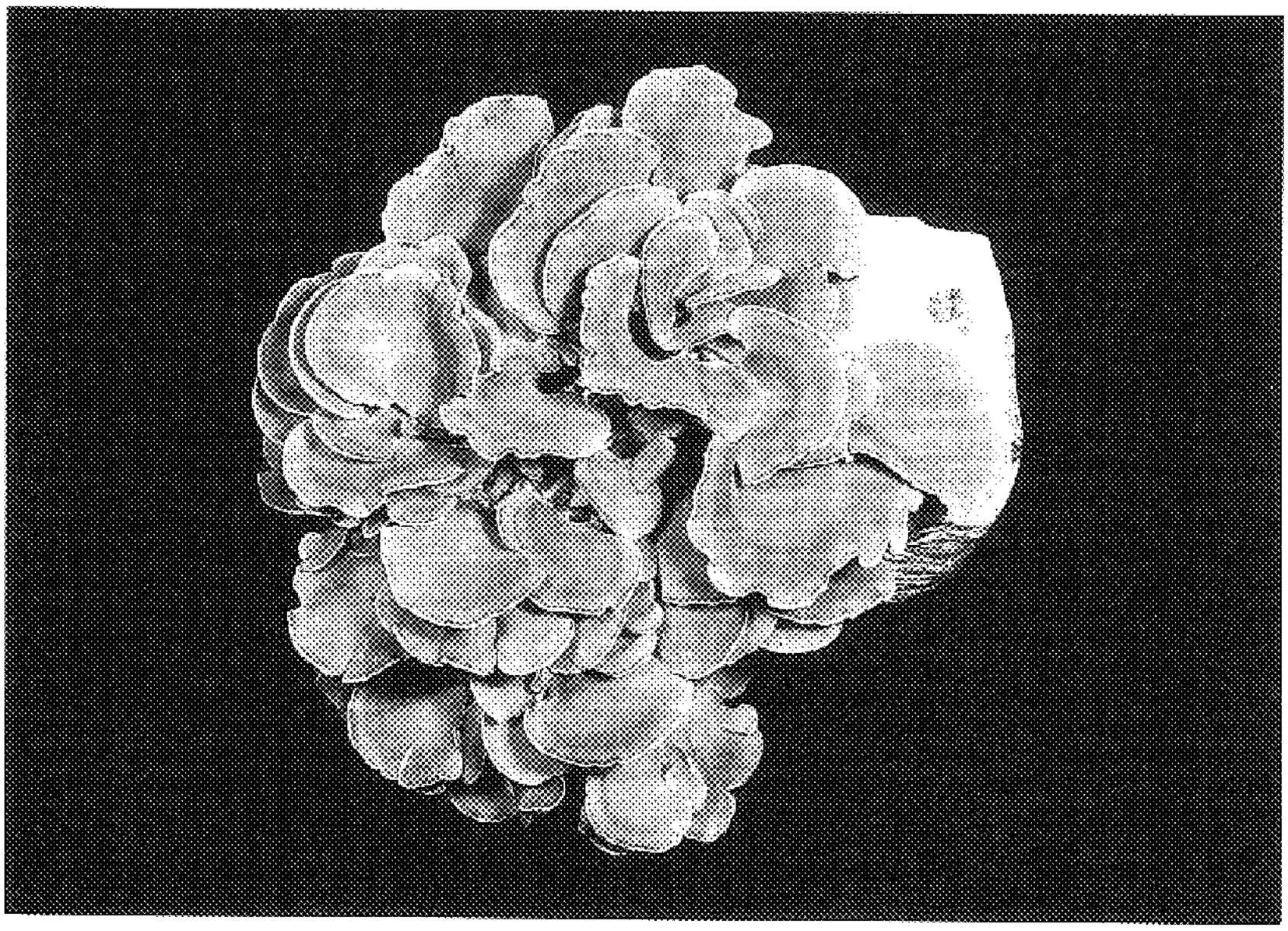


FIG. 2

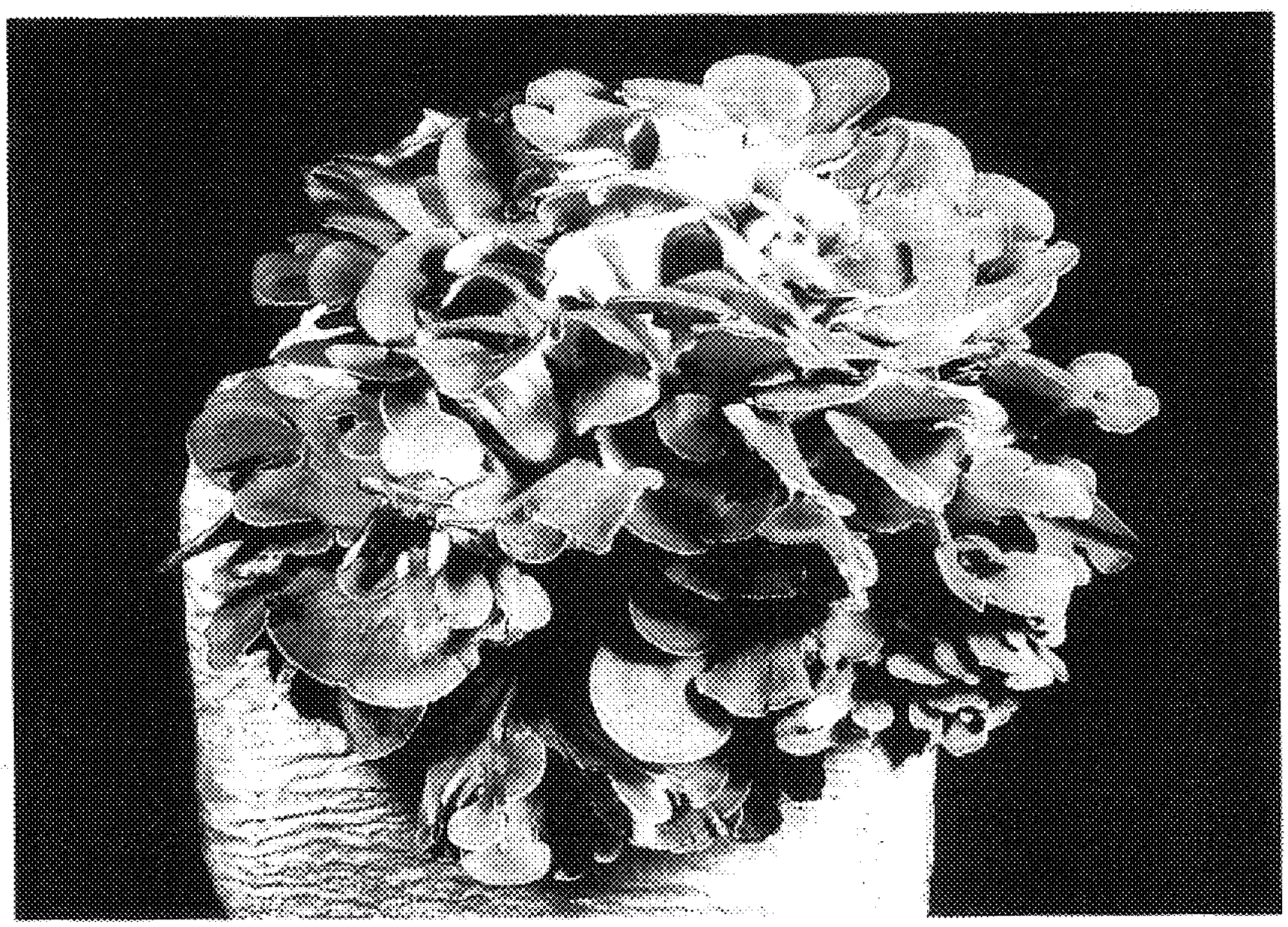


FIG. 3

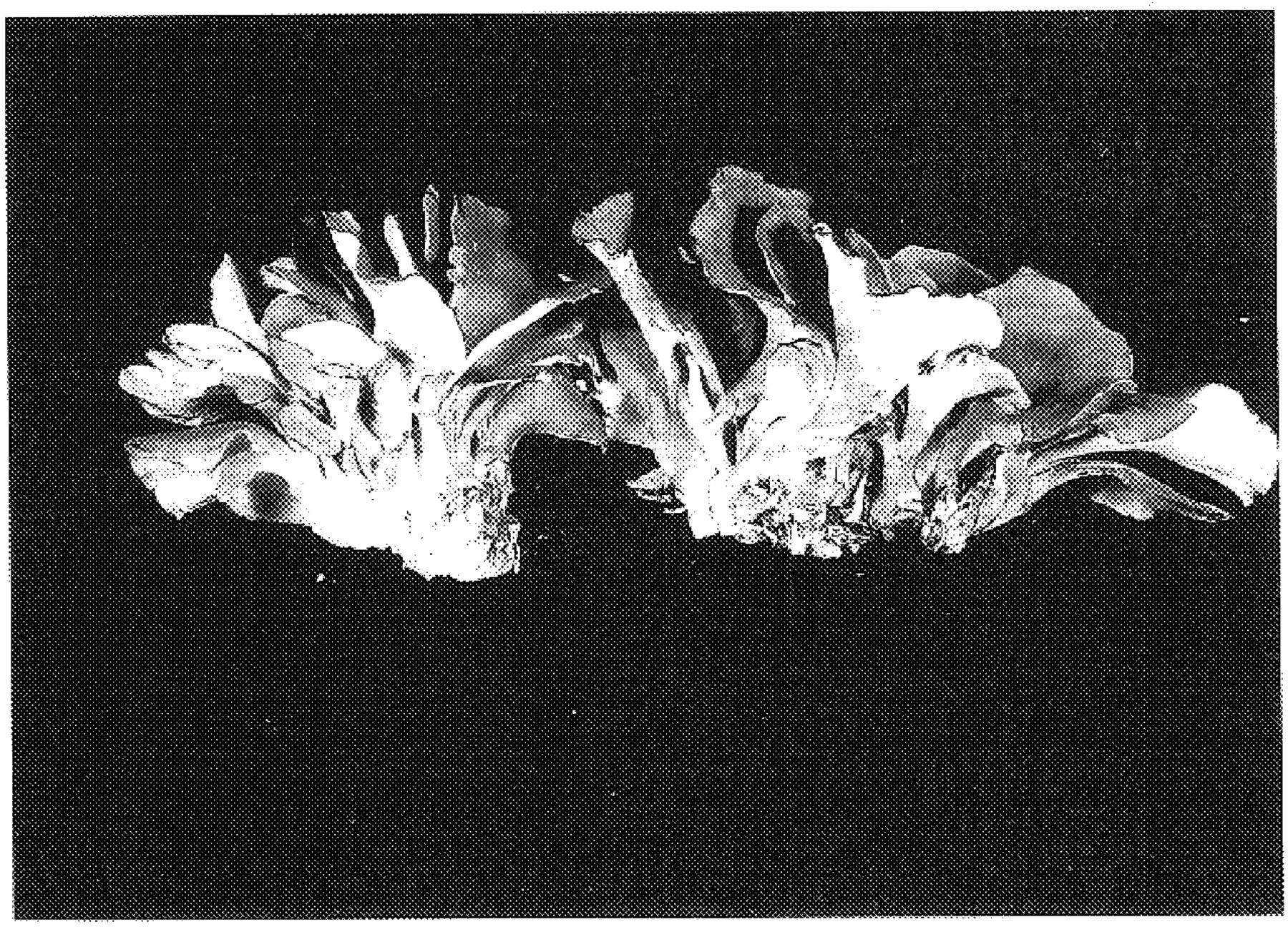


FIG. 4

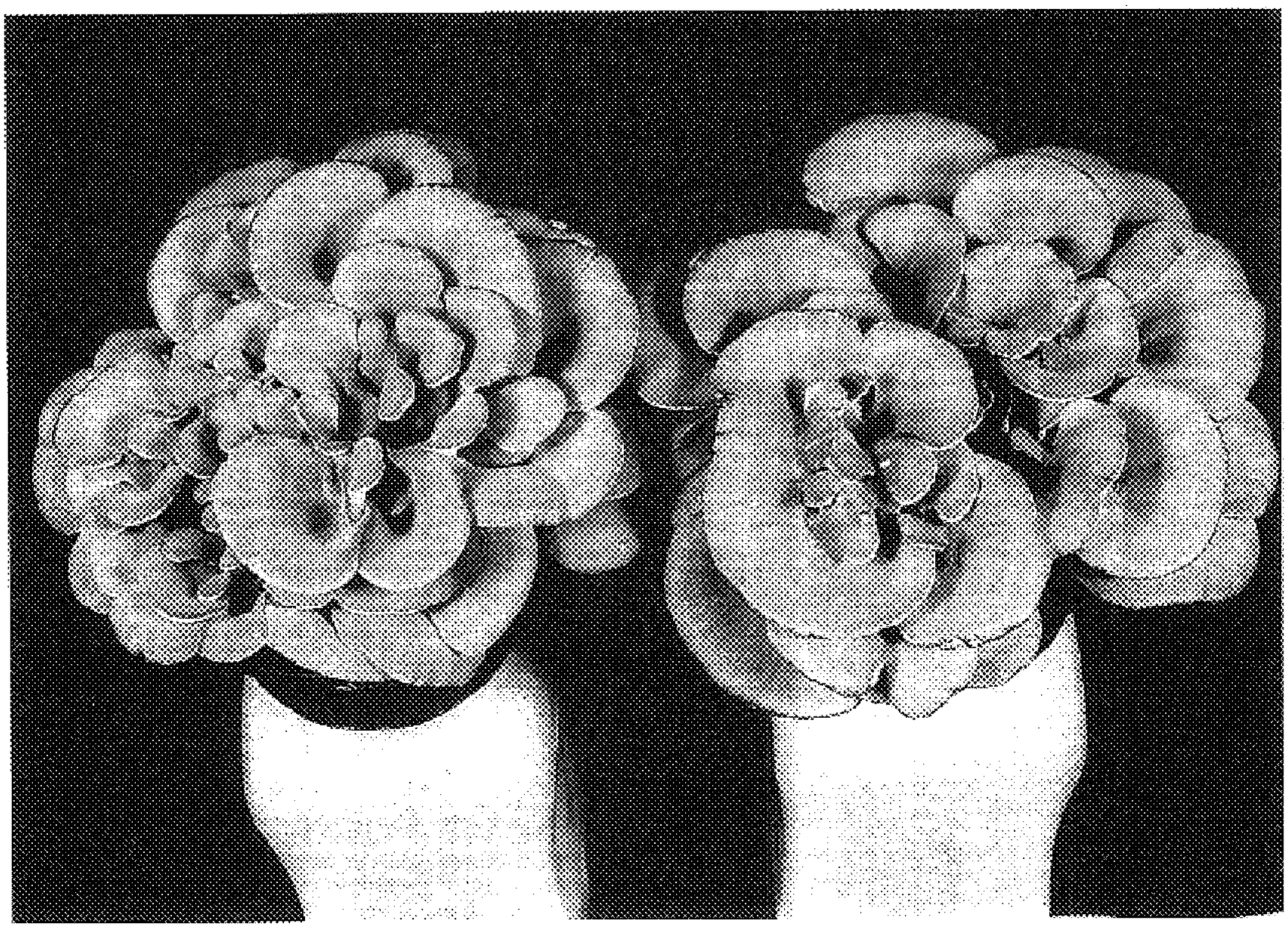


FIG. 5

FIG. 6

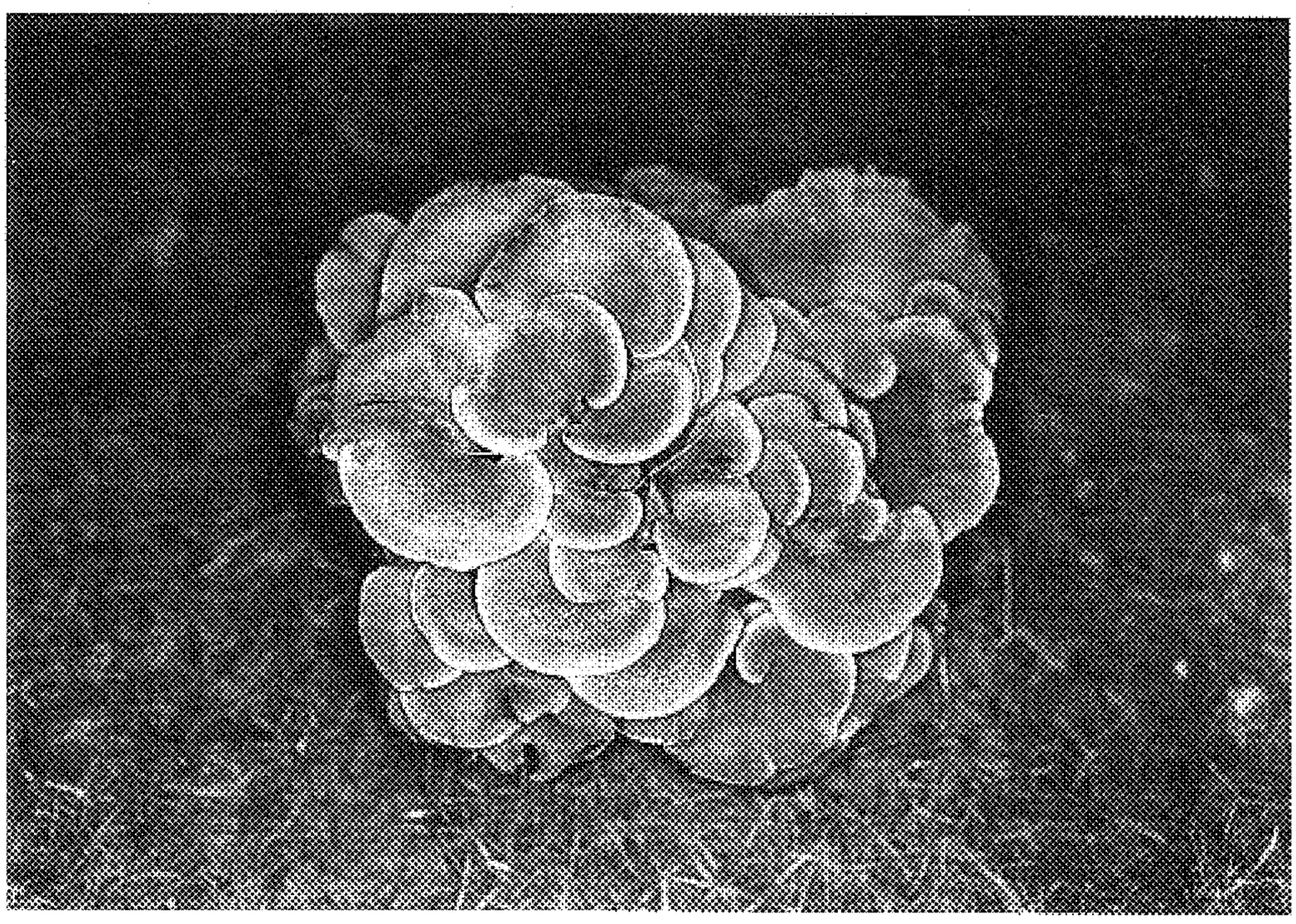


FIG. 7

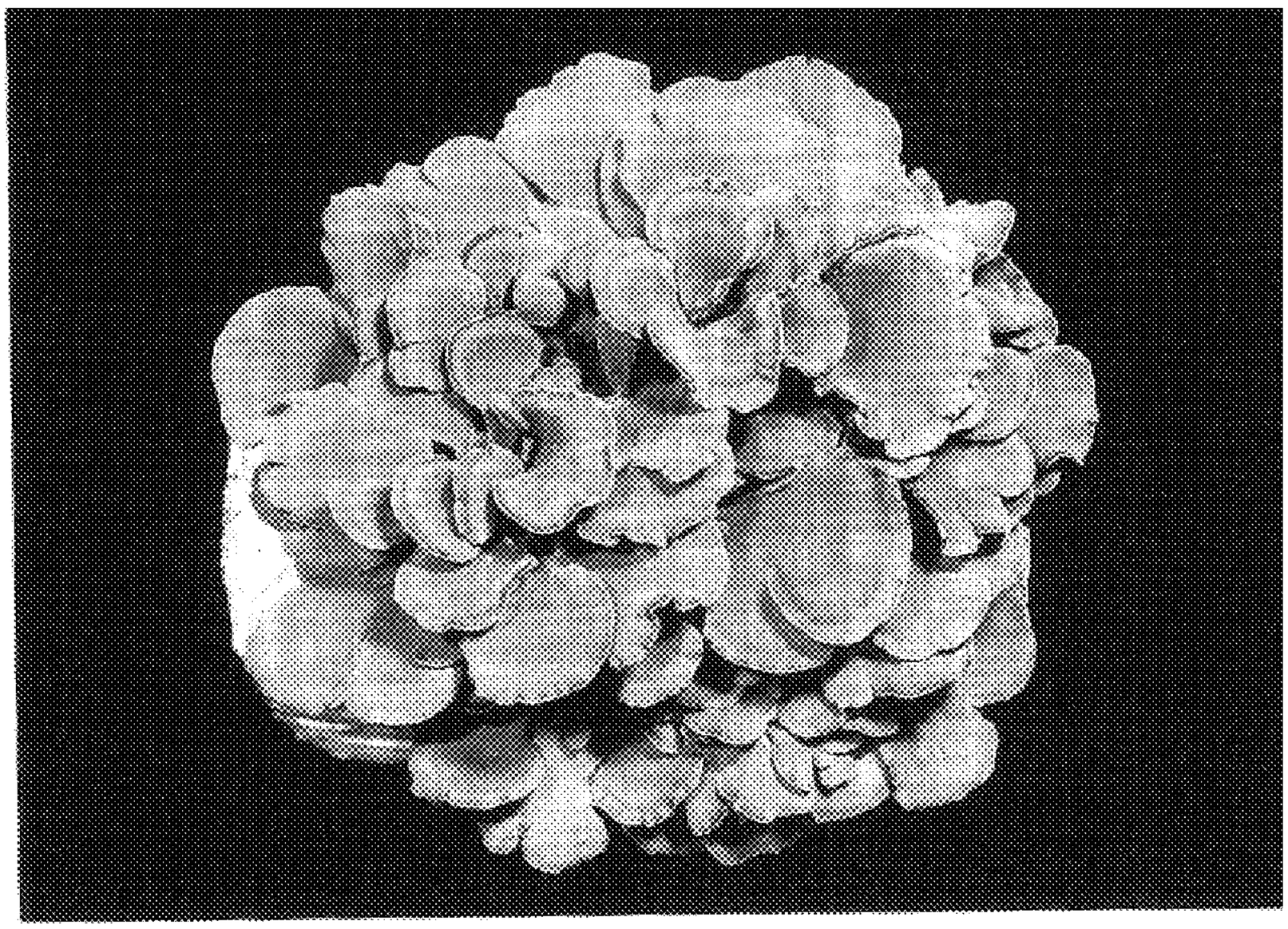


FIG. 8

FIG. 9

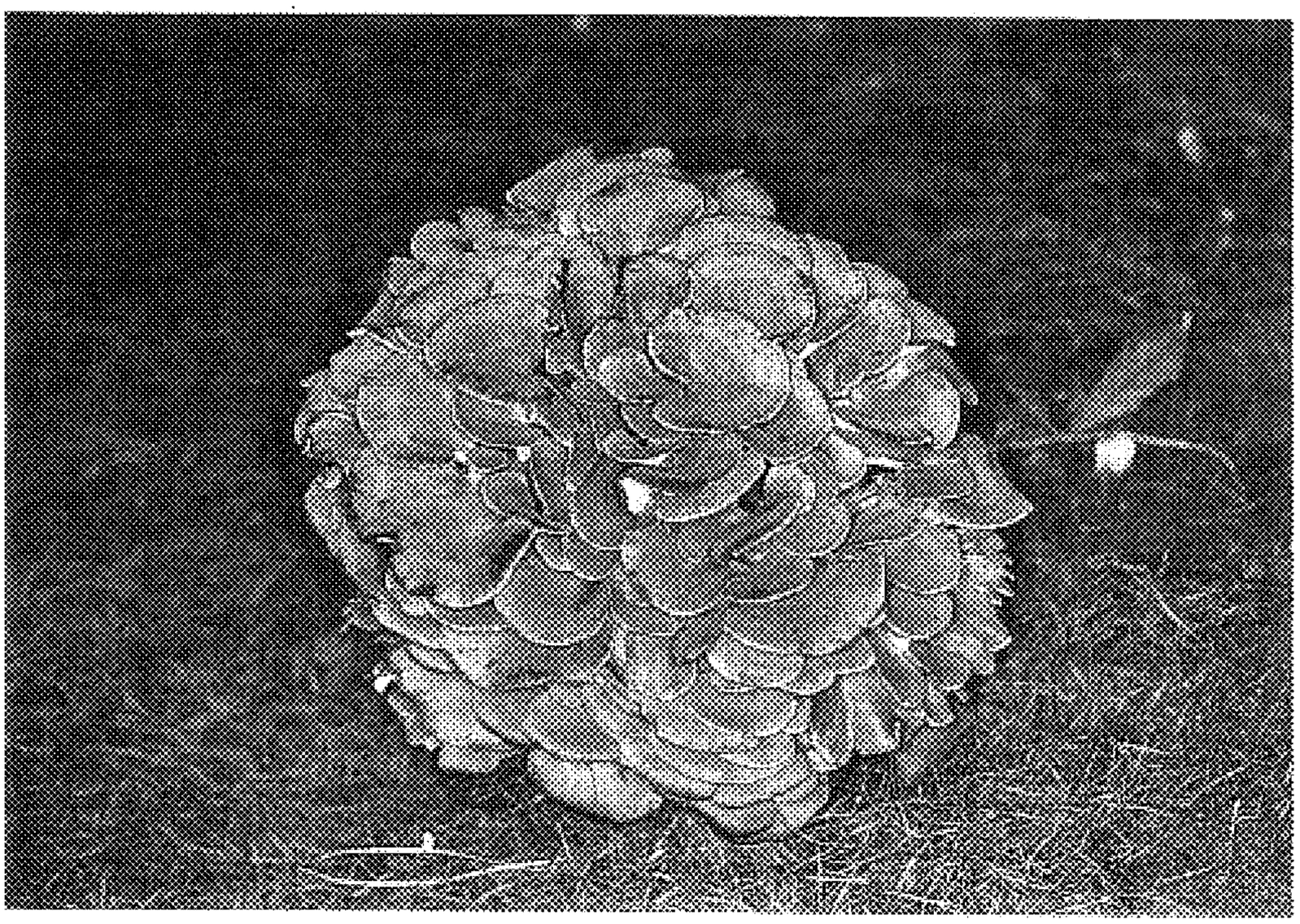


FIG. 10

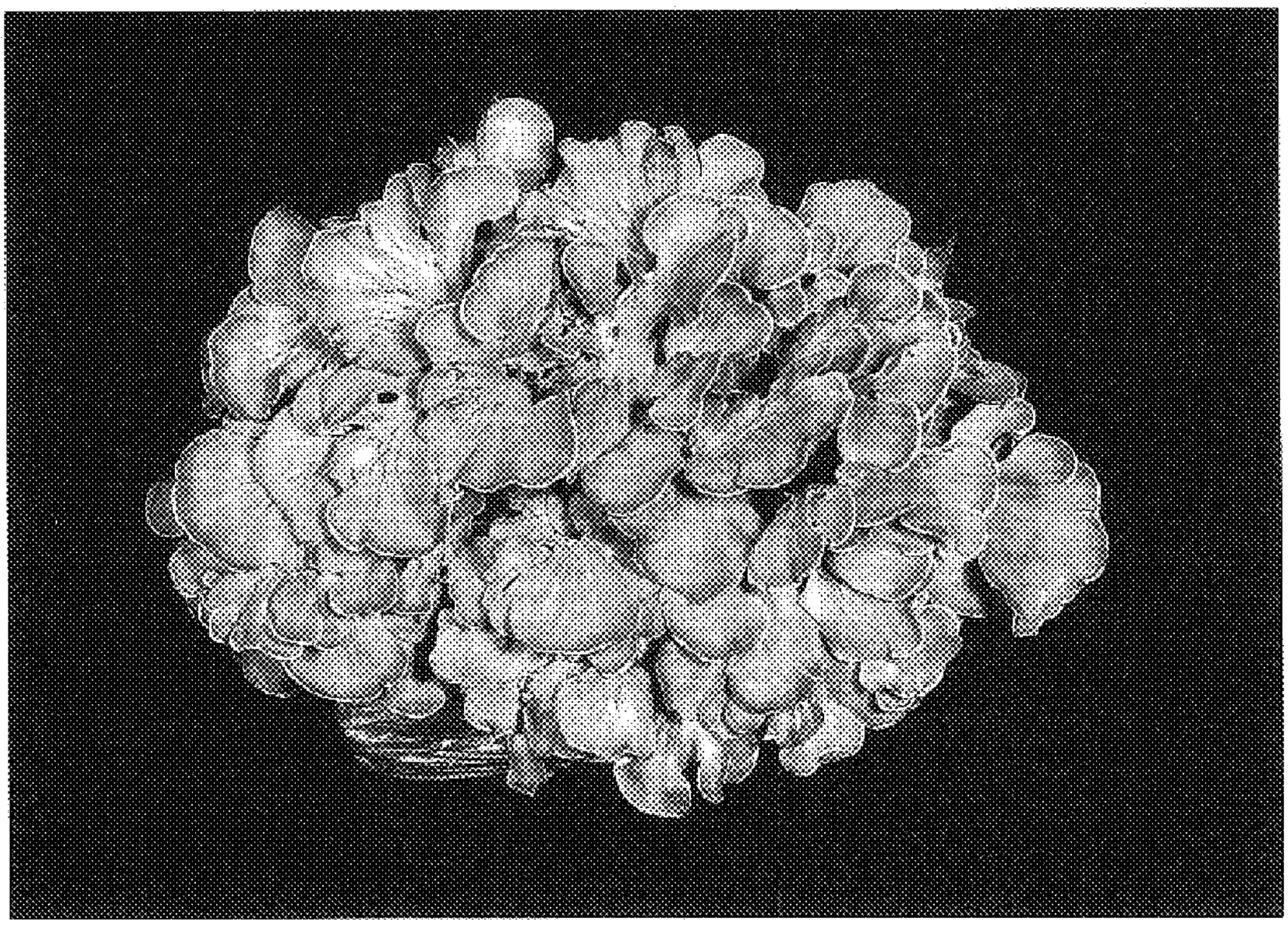


FIG. 11

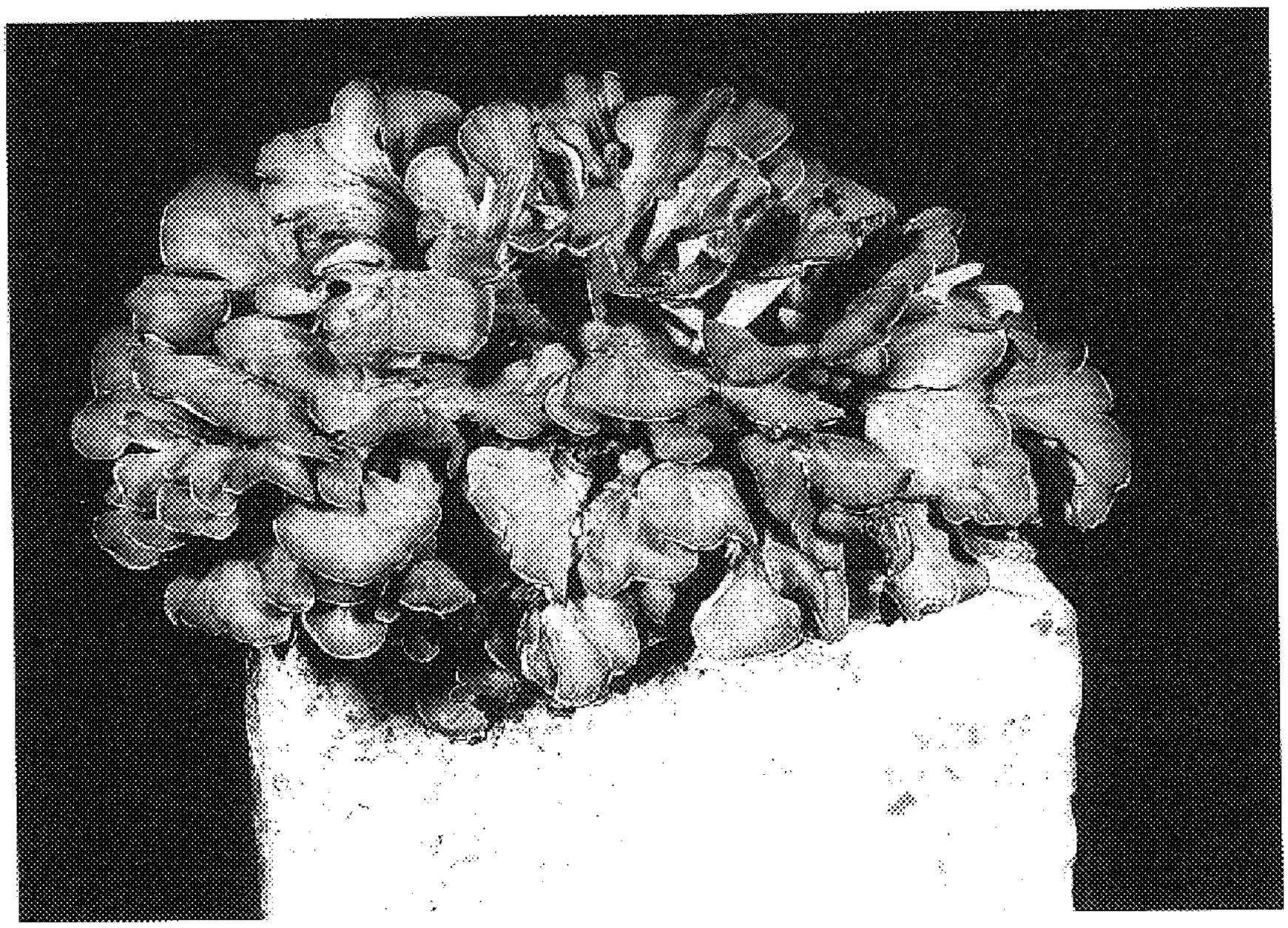


FIG. 12

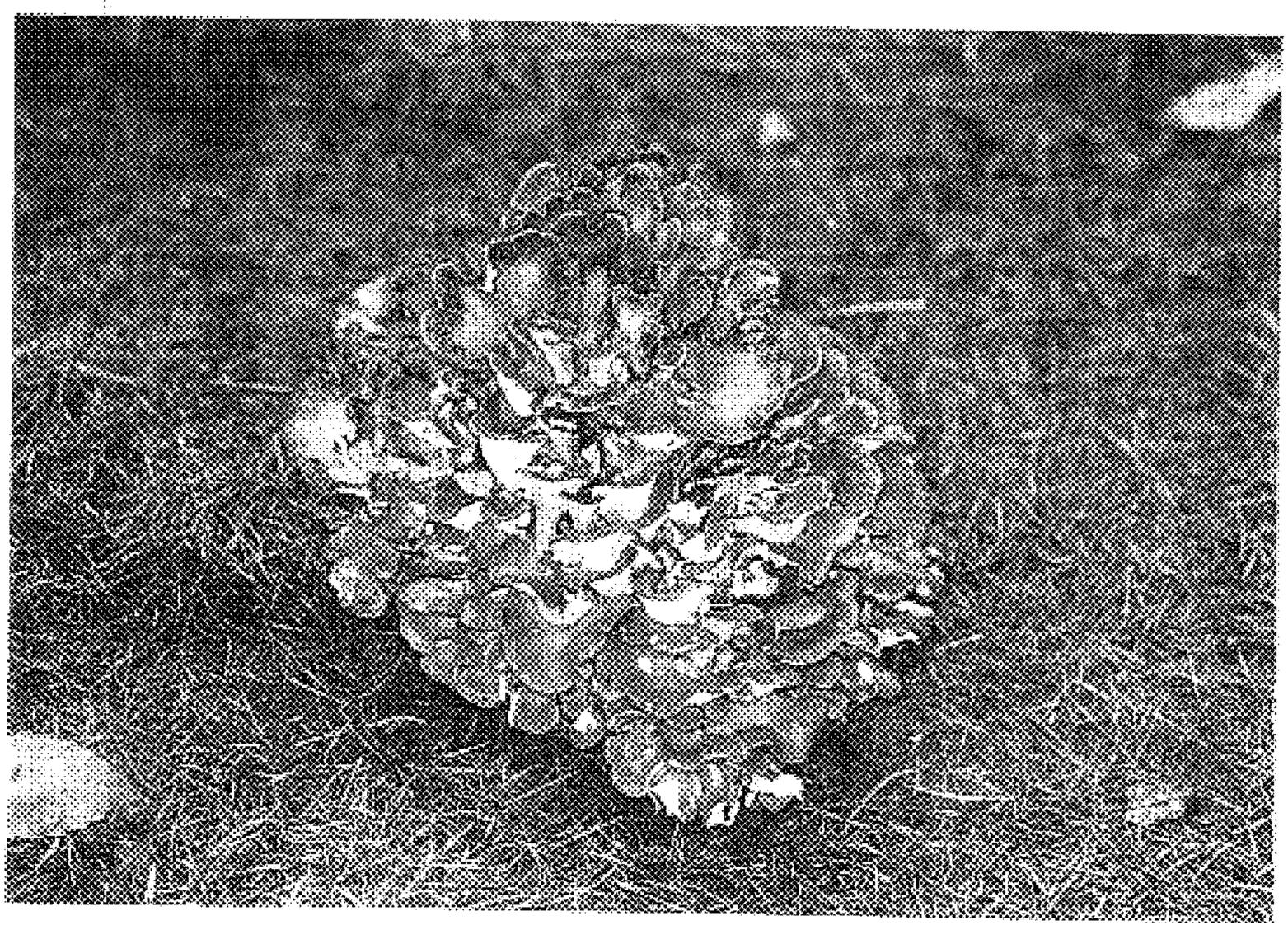
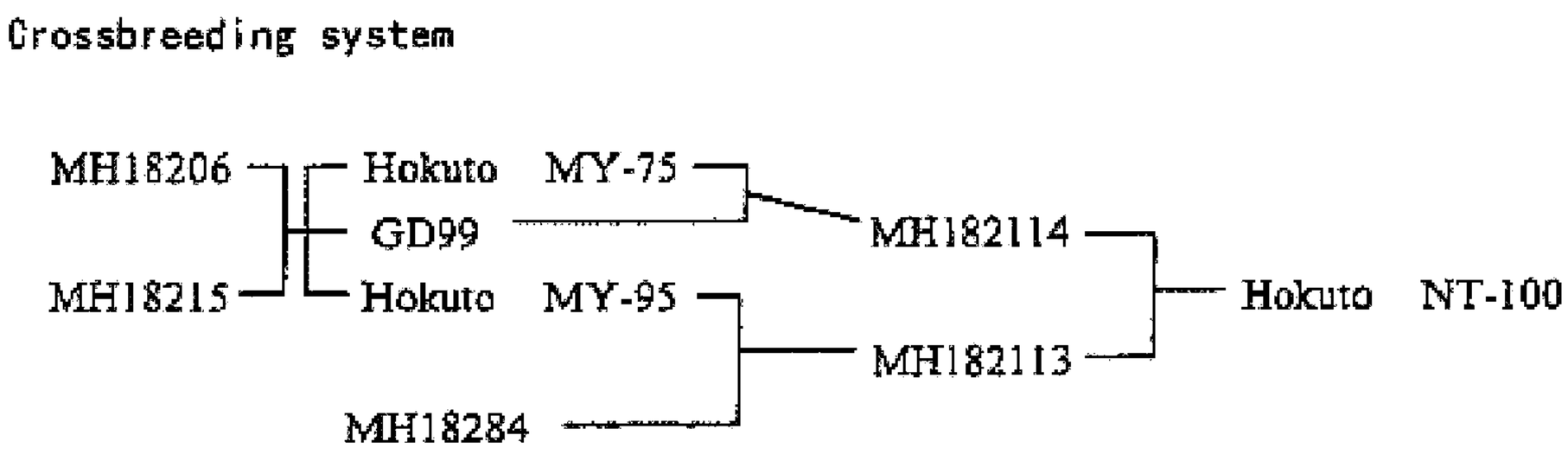


FIG. 13


Crossbreeding system
MH18206
MH18215
Hokuto
GD99
Hokuto
MY-75
MY-95
MH18284
MH182114
MH182113
Hokuto
NT-100